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## SHORT COMMUNICATION

**Identifying energy constraints to parasite resistance**

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*Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, UK***Keywords:**

costs;  
*Daphnia*;  
immunity;  
trade-offs.

**Abstract**

Life-history theory suggests that energetically expensive traits may trade off against each other, resulting in costs associated with the development or maintenance of a particular phenotype. The deployment of resistance mechanisms during parasite exposure is one such trait, and thus their potential benefit in fighting off parasites may be offset by costs to other fitness-related traits. In this study, we used trade-off theory as a basis to test whether stimulating an increased development rate in juvenile *Daphnia* would reveal energetic constraints to its ability to resist infection upon subsequent exposure to the castrating parasite, *Pasteuria ramosa*. We show that the presumably energetically expensive process of increased development rate does result in more infected hosts, suggesting that parasite resistance requires the allocation of resources from a limited source, and thus has the potential to be costly.

**Introduction**

A significant question in the study of host–parasite coevolution is why, in the face of strong directional selection pressure for hosts that are resistant to parasites, are there still susceptible genotypes (Anderson & May, 1982). One plausible explanation is that parasite resistance is costly, in that the maintenance or deployment of the resistance machinery requires the reallocation of limited internal resources away from other fitness-related traits (Stearns, 1992). Activating this machinery will therefore only be beneficial if investment in resistance is more important to fitness than other traits that trade-off against resistance.

Life-history theory suggests that achieving optimal fitness in a particular environment does not necessarily mean all fitness traits are expressed at their optimum (Reznick, 1985; Roff, 1992). This is based on the idea that an organism has a limited resource (energy) pool that must be divided among traits, and increased allocation to

one trait necessitates a decreased allocation elsewhere. A standard experimental approach to test for these trade-offs is to look for negative correlations between two or more traits under various experimental conditions, as evidence that increased investment in one trait has deleterious consequences for another. This method has been successful in revealing life-history trade-offs between traits such as current and future reproduction (Landwer, 1994; Doughty & Shine, 1998), fecundity and longevity (Rose, 1984; Sgro & Partridge, 1999), and development time and body size (Nunney, 1996).

Similarly, if there is a cost to developing and deploying resistance mechanisms, trade-offs between immune responses and fitness traits are expected and should be measurable in an analogous manner (Sheldon & Verhulst, 1996; Zuk & Stoehr, 2002). An approach to the measurement of such trade-offs is to expose hosts to an immune stimulus or parasite challenge and correlate subsequent changes in fitness measures, such as reproduction, with the development of infection (Kraaijeveld & Godfray, 1997; Fellowes *et al.*, 1998; Moret & Schmid-Hempel, 2000; Norris & Evans, 2000). However, systems where the parasite or immune challenge directly affects the fitness traits of interest can confound this approach

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(Little & Killick, 2007), i.e. it may be difficult to separate the energetic cost of launching an immune response from the cost imposed by direct damage from parasite growth.

In this study, we used the *Daphnia magna* – *Pasteuria ramosa* host–parasite pair, the latter of which castrates its host upon infection. As such, this system is not easily amenable to an approach that correlates changes in fecundity with infection, because infected hosts cease to reproduce. Therefore, rather than trying to identify costs using correlations, we used the concept of energy trade-offs to ask whether experimental manipulation of energy expenditure prior to parasite exposure affects subsequent parasite resistance. The identification of this type of energy constraint would suggest that parasite resistance has the potential to be costly.

Our approach was to stimulate increased energy expenditure in juvenile *Daphnia* and test whether that had an effect on their ability to resist parasite infection. Exposure to fish kairomones (a well-established chemical cue for the threat of fish predation) stimulates an increased rate of development in juvenile *Daphnia* sp. (Stibor, 1992; Stibor & Luning, 1994; Machacek, 1995), a presumably energy-consumptive process. By first exposing juvenile *Daphnia* to fish kairomones, and then subsequently to parasite spores, we were able to test whether mounting a resistance response utilizes the same energy source as development, and whether drawing on that resource prior to parasite exposure results in a decreased ability to defend against infection.

## Methods

### Study system

*Daphnia magna* (Crustacea: Cladocera) are cyclically parthenogenic planktonic crustaceans that inhabit freshwater lakes and ponds (Peters & de Bernardi, 1987). *Pasteuria ramosa* are spore-forming bacteria that cause sterilization and premature death in *D. magna* (Ebert *et al.*, 1996). Transmission of *P. ramosa* is exclusively horizontal, achieved by spores that are released from dead hosts and picked up by *Daphnia* during filtration feeding (Ebert *et al.*, 1996). Within the host, *P. ramosa* spores germinate and develop, culminating in the formation of transmission stage spores (Ebert *et al.*, 1996).

Host individuals were hatched from ephippia (desiccation-resistant capsules containing two eggs produced through sexual reproduction) isolated from sediment collected from a small pond population on Kames East Mains Farm near Leitholm in the Scottish Borders, UK. Four individually hatched clones (genotypes) from four different ephippia were used. The *Pasteuria* spores were also obtained from the same sediment samples as the hosts. For this, random juvenile *Daphnia* were placed in shallow trays containing sediment, artificial *Daphnia*

culture medium (Klüttgen *et al.*, 1994) and a small amount of algae. They were left in the trays for 7 days at room temperature and then removed to beakers with fresh media and plentiful algae. All individuals showing infection were grown for a further 40 days to maximize growth of transmission spores, then crushed in water and mixed to form a general *P. ramosa* spore solution.

Under favourable laboratory conditions, *D. magna* readily reproduces asexually, enabling genetic lines to be replicated for experimental purposes. Twenty-four juvenile females from each of the four genotypes were placed individually in 100 mL of artificial culture medium, and taken through two generations under experiment conditions (20 °C, 12 h light, fed  $7 \times 10^6$  cells of *Chlorella* per day) to remove potential covariances because of maternal and grand-maternal effects. Individuals from the second clutch of each of these third generation maternal-lines became the experimental animals (the first clutch was discarded). Beakers were checked daily, and new-born individuals from the second clutch were collected and one individual was randomly assigned to each of the treatment groups (described elsewhere). All clutches were not born on the same day, so this allocation process, and hence all other stages of the experiment, was staggered over 4 days.

### Design and treatment groups

The four main treatment groups were Control, Fish, Parasite and Fish + Parasite. The Control and Parasite treatments were kept in artificial culture media throughout the entire experiment, whereas the Fish and Fish + Parasite treatments were kept in 'fish-kairomone media' for a set time period before being removed to artificial culture medium (Table 1).

The fish kairomone media used in the Fish and Fish + Parasite treatments was prepared from artificial *Daphnia* culture media (Klüttgen *et al.*, 1994) in which Sticklebacks were kept at a density of one fish per 4 L. Half the media was removed and replaced daily. This collected media was filtered through a 5- $\mu$ m filter before use. Fish kairomones are known to stimulate faster development to maturity, and often larger clutch sizes, in *Daphnia* (Stibor, 1992; Stibor & Luning, 1994; Machacek, 1995). *Daphnia* mature at around 6 days of age, but there is some variation in this timing. In order to ensure that any differences in response to parasite exposure between the regular media water and fish kairomone treatments were not simply an effect of systematic differences in ages or developmental stages of the exposed individuals, each of the four main treatment groups were split into two sub-groups: D6, a day 6 'absolute age' exposure group, and DOM, a day-of-maturity 'developmental stage' exposure group. The Control and Fish treatments consisted of 24 replicates per genotype (12  $\times$  D6, 12  $\times$  DOM), whereas the Parasite and Fish + Parasite

**Table 1** Experiment design with the four main treatments: Control, Fish only, Parasite only, and Fish + Parasite; the two sub-groups within each main treatment: day 6 (D6) and day of maturity (DOM) and the sample size (*n*) per genotype.

Treatment group	<i>n</i>	Timing of treatments			
		Day of birth	Day 6	DOM	D6 or DOM + 48 h
Control	12	Water	Water + crushed <i>Daphnia</i>	Water + crushed <i>Daphnia</i>	Water
	12	Water			Water
Fish only	12	Fish water	Water + crushed <i>Daphnia</i>	Water + crushed <i>Daphnia</i>	Water
	12	Fish water			Water
Parasite only	24	Water	Water + parasite	Water + parasite	Water
	24	Water			Water
Fish + Parasite	24	Fish water	Water + parasite	Water + parasite	Water
	24	Fish water			Water

Each individual was placed in either standard media or fish media at birth. It was kept in that until either Day 6 or its DOM. It was then placed in standard media and given either crushed healthy *Daphnia* or parasite spores, for 48 h, after which it was placed in fresh standard media for the remainder of the experiment.

treatments consisted of 48 replicates per genotype (24 × D6, 24 × DOM) (Table 1).

On the day of the second clutch, six individuals from each maternal-line clutch were placed singly in 200-mL beakers and assigned to one of eight treatments. Note that only six individuals per clutch were used, because the Control and Fish treatments had half as many replicates as the Parasite and Fish + Parasite treatments (Table 1). The Control and Parasite treatments were kept in 100 mL artificial culture medium, and the Fish and Fish + Parasite treatments kept in 100 mL of fish water, until they were either 6 days old or until they were seen to be carrying eggs in their brood chamber (maturity), depending on their treatment-group assignment. All beakers were checked and fed daily and given fresh media or fish water every second day.

On their respective treatment day (Day 6 or DOM), individuals were moved singly to beakers with 50 mL artificial culture media, fed a small amount of algae ( $3 \times 10^6$  cells *Chlorella*) and given either 20 000 *P. ramosa* spores (Parasite and Fish + Parasite), or the equivalent volume of crushed healthy *Daphnia* (Control and Fish). After 2 days under parasite 'exposure' conditions, all individuals were placed singly in beakers with 100 mL of artificial culture media and fed with  $7 \times 10^6$  cells *Chlorella*. Animals were then kept for a further 20 days, fed with  $7 \times 10^6$  cells *Chlorella* daily and given fresh culture media every second day.

### Data collection

For all individual *Daphnia*, we recorded their age at maturity, number of offspring in first and second clutches, size at maturity and infection status. The number of offspring counted from the first two clutches was combined to form a single 'fecundity' variable for analysis. Offspring were discarded after counting. Body size was measured in millimetres using a Leica dissecting microscope (Leica Microsystems, Wetzlar, Germany).

### Analysis

We initially tested the three life-history traits, age at maturity, size at maturity and fecundity, for differences between the D6 and DOM groups within each of the four treatments. We included all eight groups (D6 and DOM for each of the four treatments) in an ANOVA [Proc GLM, (SAS, 2000)] and used subsequent independent contrasts to test within each of the treatments. For example, a contrast between the Fish-D6 and Fish-DOM tests for variation in responses between exposure to fish kairomones until day 6 and exposure until DOM. We found no differences between the D6 and DOM groups for any of the response variables and so combined the two sub-groups within each of the four main treatments for all subsequent analyses.

Treatment effects on the three life-history traits were analysed using ANOVA [Proc GLM, (SAS, 2000)] with the model 'trait ~ treatment + genotype + treatment × genotype'. When the main effect of 'Treatment' was significant, we used contrasts [Contrast, (SAS, 2000)] to test specifically for fish effects: (Control and Parasite) vs. (Fish and Fish + Parasite) tests the overall effect of exposure to fish kairomones relative to no fish. When the main effect of 'Treatment' was significant but not explained by the first contrast tested, we used additional post-hoc contrasts: Control vs. Fish and Parasite vs. Fish + Parasite, which test for the effect of fish exposure but allow for differences between the parasite and no-parasite treatments, and Control vs. Parasite and Fish vs. Fish + Parasite, which test for a parasite effect but allow for differences between the fish and no-fish treatments. Bonferroni adjustments for multiple testing were applied to the post-hoc contrasts prior to assigning significance, and only significant contrasts are reported in Table 2.

The effect of the exposure to fish kairomones on subsequent parasite resistance was analysed using a logistic regression [Proc Genmod, (SAS, 2000)] with a

**Table 2** Analysis of the effects of exposure to fish kairomones and parasites on age at maturity, body size at maturity and fecundity (ANOVA), and resistance to parasites (Logistic regression). Contrast analyses are listed in italics after the ANOVA results.

Source	d.f.	F	P value
<b>Age at maturity</b>			
Treatment	3	6.84	0.0002
Genotype	3	55.59	< 0.0001
Treatment × genotype	9	1.16	0.32
<i>Contrast: C&amp;P vs. F&amp;FP</i>	<i>1</i>	<i>11.86</i>	<i>0.0006</i>
<b>Size at maturity</b>			
Treatment	3	0.36	0.78
Genotype	3	25.18	< 0.0001
Treatment × genotype	9	2.19	0.022
<b>Fecundity</b>			
Treatment	3	4.27	0.005
Genotype	3	56.14	< 0.0001
Treatment × genotype	9	8.27	< 0.0001
<i>Contrast: C vs. F</i>	<i>1</i>	<i>4.86</i>	<i>0.028</i>
<i>Contrast: C vs. P</i>	<i>1</i>	<i>12.14</i>	<i>0.0005</i>
<b>Proportion of hosts infected</b>			
Treatment	1	22.36	< 0.0001
Genotype	3	33.84	< 0.0001
Treatment × genotype	3	3.22	0.36

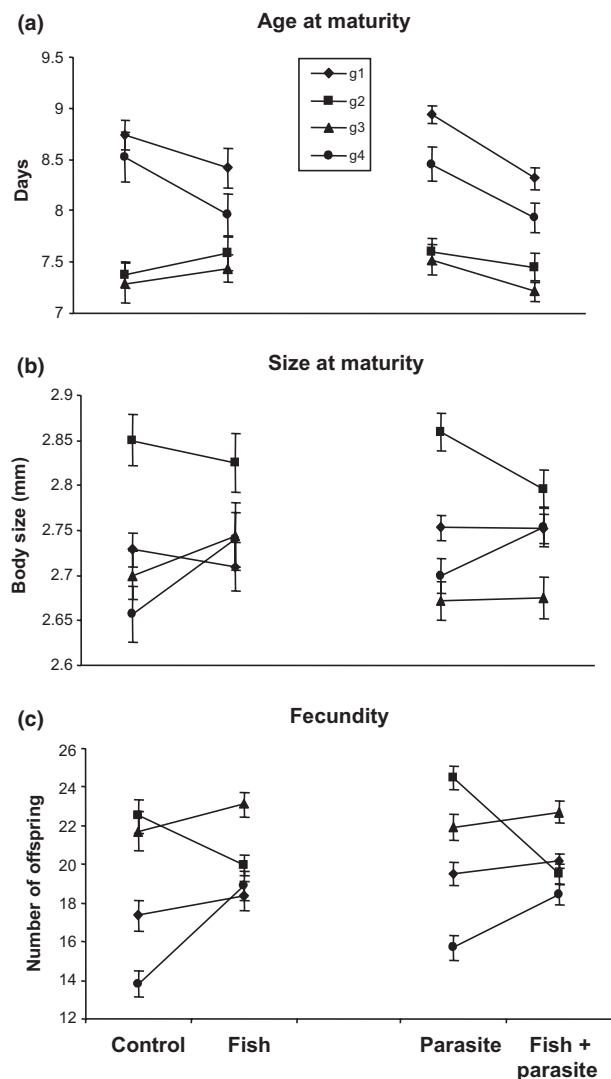
binomial distribution and logit link function. The model structure is the same as with the life-history traits using infection status as the dependent variable, but using only data from the two parasite-exposed treatment groups.

## Results

Both age at maturity and fecundity differed significantly among treatment groups, whereas treatment had no significant main effect on body size at maturity (Table 2, Fig. 1). The contrasts between fish and no-fish treatments show that exposure to fish kairomones accounts for the difference between treatments in age at maturity, with fish-exposed hosts maturing earlier (Table 2, Fig. 1a). However, the significant effect of treatments on fecundity appears to be driven by both fish and parasite effects, with the control differing significantly from both the fish-only (approaching significance after correction for multiple testing) and parasite-only treatments (Table 2, Fig. 1c).

There was a significant effect of host genotype on all three measured life-history traits (Table 2). However, for body size and fecundity, there was also a significant interaction between genotype and treatment. For fecundity at least, this interaction appears to largely be driven by host genotype 2 differing from the other three genotypes in its response to fish exposure (Fig. 1c).

Exposure to fish kairomones resulted in a significant increase in the proportion of hosts infected (Table 2, Fig. 2). There were significant differences between genotypes in the proportion infected (Table 1), but no interaction effect between genotype and treatment,



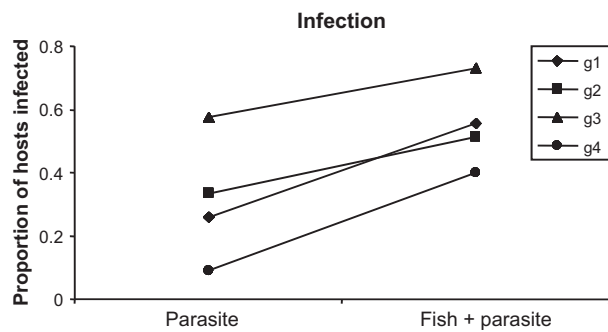
**Fig. 1** Mean life-history traits (a) age at maturity, (b) size at maturity and (c) fecundity, for each treatment group and genotype. The x-axis for each panel has the standard media treatments on the left (Control and Parasite) and fish media treatments (Fish and Fish + Parasite) on the right. Vertical bars represent two standard errors.

showing that the decreased resistance subsequent to fish exposure was consistent across all four host genotypes (Fig. 2).

## Discussion

In this study, we measured three life-history traits in order to test whether our host genotypes responded to fish kairomones as predicted by previous studies. We tested the assumption that exposure to fish kairomones stimulates an increased rate of development, a presumably energetically consumptive process, and found that





**Fig. 2** Proportion of replicates of each host genotype infected with *Pasteuria ramosa* in the two parasite-exposed treatments. The x-axis shows the standard media treatment (Parasite) on the left and fish media treatment (Fish + Parasite) on the right. The lines connect the proportion infected for each genotype in the two treatments.

*Daphnia* in the fish-exposed treatments did develop to maturity significantly faster than the unexposed treatments. While there was some variation in the magnitude of this effect among genotypes, there was no interaction between treatment and genotype indicating that the direction of rate change was consistent. This suggests that the general response of these *Daphnia* to fish kairomones is an increased investment in development rate, making this a suitable stimulus for this experiment.

Previous studies have suggested that exposure to fish kairomones may also result in a decreased body size at maturity (Weider & Pijanowska, 1993; Stibor & Luning, 1994). Although we found no overall treatment effect on body size (Table 2), there was a genotype by treatment interaction, and our largest bodied clone (genotype 2) did respond to fish kairomones with a reduction in body size and smaller clutch sizes (Fig. 1b, c). However, the remaining three genotypes tended towards larger size and increased fecundity with fish exposure, suggesting that the effect on clone 2 is probably a genotype-specific effect and not an indication that the fish treatments were directly harmful or resulted in a reduced nutrient intake.

Interestingly, there was a significant treatment effect on fecundity that was not because of exposure to fish kairomones (Table 2). Instead, individuals in the standard water treatment showed an increase in fecundity when exposed to parasites. Increased reproduction after parasite exposure has been seen previously in this system (fecundity compensation, see Ebert *et al.*, 2004), but it is interesting that the same pattern was not seen in the fish water treatments. Although speculative and not the focus of this study, this suggests that in the fish treatments, the increased energy allocation to juvenile development rate may trade off against the ability to subsequently increase reproductive investment upon parasite exposure.

Accepting that exposure to fish kairomone does stimulate increased development rate in *D. magna*, and that this requires an increased allocation of resources to the

development process, we turn to the question of how this affects resistance to parasites and the implications of any such effect. We show that *Daphnia* exposed to fish kairomones do indeed have a significantly reduced ability to resist infection when subsequently exposed to the bacterial parasite, *P. ramosa* (Table 2, Fig. 2). Although there are significant genetic differences in the hosts' ability to resist *Pasteuria* (Table 2), there was no variation in the direction of the effect of fish exposure: all four host genotypes had increased infection levels relative to the same genotypes in the no-fish treatment (Fig. 2). This result suggests that the modification of development time in response to the predator cue has apparently required the allocation of resources that would otherwise be used for parasite resistance.

It is possible that there are trait responses to predator cues that directly affect susceptibility independent of energy expenditure. *Daphnia magna* have been shown to respond to fish cues by altering traits as varied as eye diameter, percentage of male offspring, and phototactic behaviour (Boersma *et al.*, 1998). For many such traits (e.g. eye size), it is not immediately obvious how this could directly increase susceptibility, though the possibility cannot be rejected. Additionally, unlike some other *Daphnia* species, *D. magna* do not produce any obvious defence structures such as neck teeth (Lüning, 1992) or 'crown of thorns' (Petrusek *et al.*, 2009) that could conceivably impact susceptibility by, for example, enlarging the host surface area. Thus, although our energy limitation hypothesis is not the only possible explanation, it is certainly among the most parsimonious.

Previous studies have found costs of mounting an immune response only under low food conditions (Moret & Schmid-Hempel, 2000; McKean & Nunnery, 2005) and argue that trade-off costs may not be seen if there are sufficient resources available for compensatory resource intake (Moret & Schmid-Hempel, 2000). The present experiment was not performed under low food conditions. This suggests that the reduced resistance we see may actually be the consequence of underdeveloped resistance machinery, because of resource reallocation during development, rather than there being insufficient resources directly available to mount a resistance response. In support of this argument is the observation that the two genotypes with the largest increase in infection rates (~30% increase in these two genotypes compared to ~15% increase in the other genotypes) were also the two genotypes with the most dramatic decrease in development time in response to the fish kairomones (Figs 1a and 2).

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## References

- Anderson, R.M. & May, R.M. 1982. Coevolution of hosts and parasites. *Parasitology* **85**: 411–426.
- Boersma, M., Spaak, P. & Meester, L.D. 1998. Predator-mediated plasticity in morphology, life history, and behavior of *Daphnia*: the uncoupling of responses. *Am. Nat.* **152**: 237–248.
- Doughty, P. & Shine, R. 1998. Reproductive energy allocation and long-term energy stores in a viviparous lizard (*Eulamprus tympanum*). *Ecology* **79**: 1073–1083.
- Ebert, D., Rainey, P., Embley, T. & Scholz, D. 1996. Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. *Philos. Trans. R. Soc. Lond. B* **351**: 1689–1701.
- Ebert, D., Carius, H.J., Little, T. & Decaestecker, E. 2004. The evolution of virulence when parasites cause host castration and gigantism. *Am. Nat.* **164**: S19–S32.
- Fellowes, M.D.E., Kraaijeveld, A.R. & Godfray, H.C.J. 1998. Trade-off associated with selection for increased ability to resist parasitoid attack in *Drosophila melanogaster*. *Proc. R. Soc. B. Biol. Sci.* **265**: 1553–1558.
- Klüttgen, B., Dülmer, U., Engels, M. & Ratte, H.T. 1994. ADaM, an artificial freshwater for the culture of zooplankton. *Water Res.* **28**: 743–746.
- Kraaijeveld, A.R. & Godfray, H.C.J. 1997. Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature* **389**: 278–280.
- Landwer, A.J. 1994. Manipulation of egg production reveals costs of reproduction in the tree lizard (*Urosaurus ornatus*). *Oecologia* **100**: 243–249.
- Little, T.J. & Killick, S.C. 2007. Evidence for a cost of immunity when the crustacean *Daphnia magna* is exposed to the bacterial pathogen *Pasteuria ramosa*. *J. Anim. Ecol.* **76**: 1202–1207.
- Lüning, J. 1992. Phenotypic plasticity of *Daphnia pulex* in the presence of invertebrate predators: morphological and life history responses. *Oecologia* **92**: 383–390.
- Machacek, J. 1995. Inducibility of life history changes by fish kairomone in various developmental stages of *Daphnia*. *J. Plankton Res.* **17**: 1513–1520.
- McKean, K.A. & Nunney, L. 2005. Bateman's principle and immunity: phenotypically plastic reproductive strategies predict changes in immunological sex differences. *Evolution* **59**: 1510–1517.
- Moret, Y. & Schmid-Hempel, P. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. *Science* **290**: 1166–1168.
- Norris, K. & Evans, M.R. 2000. Ecological immunology: life history trade-offs and immune defense in birds. *Behav. Ecol.* **11**: 19–26.
- Nunney, L. 1996. The response to selection for fast larval development in *Drosophila melanogaster* and its effect on adult weight: an example of a fitness trade-off. *Evolution* **50**: 1193–1204.
- Peters, R.H. & de Bernardi, R. 1987. Why *Daphnia*? In: *Daphnia*, Vol. 45 (R.H. Peters & R. de Bernardi, eds), pp. 1–9. Consiglio Nazionale Delle Ricerche, Verbania Pallanza.
- Petrusek, A., Tollrian, R., Schwenk, K., Haas, A. & Laforsch, C. 2009. A “crown of thorns” is an inducible defense that protects *Daphnia* against an ancient predator. *Proc. Natl Acad. Sci. USA* **106**: 2248–2252.
- Reznick, D. 1985. Costs of reproduction: an evaluation of the empirical evidence. *Oikos* **44**: 257–267.
- Roff, D.A. 1992. *The Evolution of Life Histories: Theory and Analysis*. Chapman and Hall, New York.
- Rose, M.R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution* **38**: 1004–1010.
- SAS 2000. *SAS OnlineDoc*®, Version 8. SAS Institute Inc, Cary.
- Sgro, C.M. & Partridge, L. 1999. A delayed wave of death from reproduction in *Drosophila*. *Science* **286**: 2521–2524.
- Sheldon, B.C. & Verhulst, S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* **11**: 317–321.
- Stearns, S.C. 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford.
- Stibor, H. 1992. Predator induced life-history shifts in a freshwater cladoceran. *Oecologia* **92**: 162–165.
- Stibor, H. & Luning, J. 1994. Predator-induced phenotypic variation in the pattern of growth and reproduction in *Daphnia hyalina* (Crustacea: Cladocera). *Funct. Ecol.* **8**: 97–101.
- Weider, L.J. & Pijanowska, J. 1993. Plasticity of *Daphnia* life histories in response to chemical cues from predators. *Oikos* **67**: 385–392.
- Zuk, M. & Stoehr, A.M. 2002. Immune defense and host life history. *Am. Nat.* **160**: S9–S22.

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